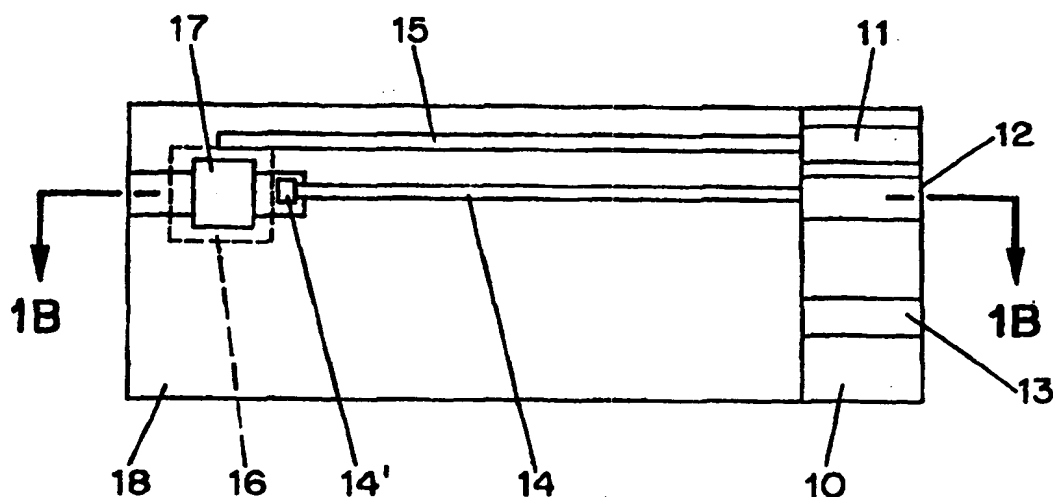


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International Bureau

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(54) Title: DISPOSABLE TEST STRIPS WITH INTEGRATED REAGENT/BLOOD SEPARATION LAYER**(57) Abstract**

An improved disposable glucose test strip for use in a test meter of the type which receives a disposable test strip and a sample of blood from a patient and performs an electrochemical analysis using a non-conductive integrated reagent/blood separation layer (17) containing a filler, an enzyme effective to oxidize glucose, e.g., glucose oxidase, and a mediator effective to transfer electrons from the enzyme. The integrated layer formulation is printed over a conductive carbon element (16) to form a working electrode. The filler, for example a silica filler, is selected to have a balance of hydrophobicity such that on drying it forms a two-dimensional network on the surface of the conductive element. The response of this test strip is essentially temperature independent over relevant temperature ranges and is substantially insensitive to the hematocrit of the patient.

DISPOSABLE TEST STRIPS WITH INTEGRATED
REAGENT/BLOOD SEPARATION LAYER

DESCRIPTION

BACKGROUND OF THE INVENTION

This application relates to disposable test strips for use in electrochemical determinations of blood analytes such as glucose, and to methods and compositions for use in making such strips.

5 Glucose monitoring is a fact of everyday life for diabetic individuals, and the accuracy of such monitoring can literally mean the difference between life and death. To accommodate a normal life style to the need for frequent monitoring of glucose levels, a number of glucose meters are now available which permit the individual to test the glucose level in a small amount of blood.

10 Many of these meters detect glucose in a blood sample electrochemically, by detecting the oxidation of blood glucose using an enzyme such as glucose oxidase provided as part of a disposable, single-use electrode system. Examples of devices of this type are disclosed in European Patent No. 0 127 958, and US Patents Nos. 5,141,868, 5,286,362, 5,288,636, and 5,437,999 which are
15 incorporated herein by reference for purposes of those countries which permit such incorporation.

20 In general, existing glucose test strips for use in electrochemical meters comprise a substrate, working and reference electrodes formed on the surface of the substrate, and a means for making connection between the electrodes and the meter. The working electrode is coated with an enzyme capable of oxidizing glucose, and a mediator compound which transfers electrons from the enzyme to the electrode resulting in a measurable current when glucose is present. Representative mediator
25 compounds include ferricyanide, metallocene compounds such as ferrocene, quinones, phenazinium salts, redox indicator DCPIP, and imidazole-substituted osmium compounds.

Working electrodes of this type have been formulated in a number of ways. For example, mixtures of conductive carbon, glucose oxidase and a mediator

5 A further challenge facing sensors for electrochemical glucose detection arises as a result of interference from blood cells present in the sample. The level of red blood cells is reflected in the hematocrit reading. Typically, high hematocrit samples results in readings that are lower than the true value, while low hematocrit samples result in readings that are higher because the blood cells tend to foul the surface of the electrode and limit electron transfer. Also, oxygen bound to the hemoglobin of red blood cells competes with the mediator for the reduced enzyme, thereby further diminishing the glucose response. Attempts have been made to limit the hematocrit effect by adding a membrane to filter out blood components (see, US Patent No. 5,658,444, which is incorporated herein by reference for purposes of those countries which permit such incorporation), but this adds an extra step to the manufacturing process, with associated increase in cost and often degraded performance in other areas such as precision.

15 Because of the importance of obtaining accurate glucose readings to the well-being of a patient using the meter and disposable test strips, it would be highly desirable to have a glucose test strip which did not suffer from these drawbacks, and which therefore provided a more consistent and reliable indication of actual blood glucose values, regardless of actual conditions. It is therefore an object of the present invention to provide disposable glucose test strips which provide a glucose reading that is essentially independent of the hematocrit of the sample, and which include an integrated reagent/blood separation layer.

20 It is a further object of the present invention to provide an improved method for making disposable glucose test strips.

25 SUMMARY OF THE INVENTION

The present invention provides an improved disposable test strip for use in a test meter of the type which receives a disposable test strip and a sample of blood from a patient and performs an electrochemical analysis of the amount of a blood analyte such as glucose in the sample. The test strip comprises:

- 30 (a) a substrate;
- (b) a first conductive element disposed on the substrate;

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A and 1B show an electrode structure useful in a disposable test strip in accordance with the invention;

Fig. 2 shows a test strip in accordance with the invention;

Figs. 3A - 3C show the current measured as a function of glucose concentration for three different hematocrit levels;

Fig. 4 shows the relationship of the glucose-concentration dependence of the measured current as a function of hematocrit;

Figs. 5A - 5C show the current measured as a function of glucose in blood and a control solution for three different conductive elements;

Figs. 6A and 6B show the current measured as a function of glucose at two different temperatures;

Fig. 7 shows a further embodiment of a glucose test strip according to the invention;

Figs 8A and 8B show current transients observed using a test strip according to the invention and a commercial carbon-based test strip;

Figs. 9A-C show a three-step process for manufacture of test strips in accordance with the invention; and

Figs. 10A-10G show the manufacture of a test strip in accordance with the invention.

DETAILED DESCRIPTION OF THE INVENTION

Figs. 1A and 1B show electrodes useful in a disposable test strip in accordance with the invention. As shown, the electrodes are formed on a substrate 10. On the substrate 10 are placed two conductive elements 14' and 16, connected by leads 14 and 15 to conductive contacts 11, 12, and 13. An insulating mask 18 is then formed, leaving at least a portion of conductive elements 14' and 16, and the contacts 11, 12 and 13 exposed. A non-conductive integrated reagent/blood separation layer 17 is then applied over the insulating mask 18 to make contact with conductive element 16.

methodology allows the formation of the test strip in only three steps. In the first step (Fig. 9A), two conductive elements 14' and 16 and associated leads and contacts are deposited on a substrate. In a second step (Fig. 9B), a layer of insulating material is deposited over the conductive elements. The insulating material has two apertures 94 and 96, one in alignment with each of the conductive elements 14' and 16. In the third step, (Fig. 9C), the integrated reagent/blood separation layer 17 is deposited over the aperture 96. By making the deposited layer 17 larger in dimensions than the aperture 96, the reagent layer completely covers the underlying conductive element such that it is not exposed directly to the sample, thereby providing effective blood separation.

The complete coverage of conductive element 16 also addresses another source of error which can occur as a result of electrochemical oxidation or small molecules such as ascorbic acid, uric acid and acetaminophen which may be present in the sample. When present, the oxidation of these molecules at the surface of the electrode leads to spuriously elevated current levels, and thus an inaccurate measurement of the desired analyte, e.g. glucose. The integrated reagent/blood separation layer of invention will not generally exclude these molecules, since they are small compared to the pore sizes observed. However, by including a pH buffer in the integrated reagent/blood separation layer one can shift the local pH at the electrode surface to a level where electrochemical potential of these species is higher. Thus, for example, the use of an integrated reagent/blood separation layer in which the pH is buffered to a level of around pH 5 will substantially reduce the impact of these interferents. To maximize the effectiveness of this buffering, however, the entire conductive element must be covered, since even a relatively small region of exposed (not buffered) electrode surface can result in a large interference current.

Not only do the test strips of the invention provide performance benefits resulting from the separation of the conductive element from the blood sample, the test strips of the invention are also resistant to other sources of error. For example, during the period of a test, reagents may diffuse laterally away from the original deposit. If the reagent layer is deposited directly on the conductive element, these reagents will continue to contribute to the measured signal. Any variations in

polyester film, and other insulating substrate materials such as polyvinyl chloride (PVC) and polycarbonate.

The conductive elements and associated leads and contacts can be formed from essentially any conductive material including silver, Ag/AgCl, gold, or platinum/carbon, and need not all be formed from the same material. The conductive element 16 is preferably formed from conductive carbon. Preferred conductive carbon are ERCON ERC1, ERCON ERC2 and Acheson Carbon Electrodag 423. Carbon with these specifications is available from Ercon Inc. (Waltham, Massachusetts, USA), or Acheson Colloids, (Princes Rock, Plymouth, England). The conductive element 16 makes contact with working electrode track 15, and is close to, but not contacting conductive element 14' disposed as the end of reference electrode track 14.

The insulating layer 18 can be formed from polyester-based printable dielectric materials such as ERCON R488-B(HV)-B2 Blue. The top cover 23 is suitably formed from a polyester strip or a "hot melt" coated plastic.

The test strips of the present invention do not require the formation of a discrete exit port to permit air to escape from the device as sample enters the electrode chamber but instead uses a distributed exit along all of the edges of the mesh. As the sample fluid wicks along the mesh, air seeps out of the edges of the mesh all around the device underneath the top layer. The sample fluid does not seep out because the insulation layer imparts significant hydrophobicity to that part of the mesh. The liquid sample therefore remains in the central hydrophilic region.

The key to the performance achieved using the present invention is in the nature of the integrated reagent/blood separation layer 17. This layer can be formed from a mixture containing a filler which has both hydrophobic and hydrophilic surface regions, and in the case of a glucose test strip, an enzyme which can oxidize glucose, and a mediator which can transfer electrons from the enzyme to the underlying conductive element layer 16. This layer is suitably formed by formulating an ink which contains the filler, the enzyme and the mediator in a suitable carrier and using this ink to print the layer 17 onto the device.

Furthermore, the gelled reaction zone presents a greater barrier to entry of blood analytes such as glucose which makes the device diffusion, rather than kinetically limited. This leads to a device in which the measured current varies by less than 10 percent over a temperature range from 20°C to 37°C and which is thus essentially temperature independent. (Figs. 6A and 6B)

When making a glucose test strip, the integrated reagent/blood separation layer is advantageously formed from an aqueous composition containing 2 to 10 % by weight, preferably 4 to 10 % and more preferably about 4.5 % of a binder such as hydroxyethylcellulose or mixtures of hydroxyethylcellulose with alginate or other thickeners; 3 to 10 % by weight, preferably 3 to 5 % and more preferably about 4 % silica; 8 to 20 % by weight, preferably 14 to 18 % and more preferably about 16 % of a mediator such as ferricyanide; and .4 to 2 % by weight, preferably 1 to 2 % and more preferably about 1.6 % of an enzyme such as glucose oxidase, assuming a specific activity of about 250 units/mg, or about 1000 to 5000 units per gram of ink formulation.

The integrated reagent/blood separation layer may also include additional ingredients without departing from the scope of the invention. For example, the nonconducting layer may include an antifoam. In addition, the nonconducting layer may be formulated with a buffering agent to control the pH of the reaction zone. The pH may be maintained at a level within the range from about pH 3 to pH 10. In one embodiment of the invention, it is of particular utility to maintain the pH of the device at a level above 8 because at this pH oxygen bound to hemoglobin is not released. Further, at this pH, the reaction rate of glucose oxidase with oxygen is very low. Thus, selection of an appropriate pH can further stabilize the performance of the test strip against the effects of varying hematocrit. In an alternative embodiment of the invention, maintaining a low pH (below pH 5.5, the optimum pH for reaction of glucose oxidase with oxygen) may be preferred. For example, maintaining a pH of around pH 5 is better if the primary concern is the elimination of electrochemical interferences arising from oxidation of interfering substances such as ascorbic acid, uric acid or acetaminophen, since these compounds are more difficult to oxidize at lower pH.

Units/mg) was added and then thoroughly mixed into the solution. The resulting formulation was ready for printing, or could be stored with refrigeration.

EXAMPLE 2

5 To prepare glucose test strips using the ink formulation of Example 1, a series of patterns are used to screen print layers onto a 330 micron polyester substrate (Melinex 329). The first step is the printing of carbon pads. An array of 10 X 50 pads of carbon is formed on the surface of the polyester substrate by printing with EC2 carbon. (Ercon) The printed substrate is then passed through a heated dryer, and optionally cured at elevated temperature (e.g. 70°C) for a period of 1 to 3 weeks.

10 Next, an array of silver/silver chloride connecting tracks and contacts is printed onto the substrate using ERCON R-414 (DPM-68)1.25 bioelectrode sensor coating material and dried. One working track which makes contact with the carbon pad and one reference track is printed for each carbon pad in the array.

15 A dielectric layer is then printed using ERCON R488-B(HV)-B2 Blue and dried. The dielectric layer is printed in a pattern which covers substantially all of each device, leaving only the contacts, the tip of the reference electrode and the carbon pads uncovered.

20 On top of the dielectric layer the ink of Example 1 is used to form an integrated reagent/blood separation layer overlaid on top of each conductive carbon pad.

25 Polyester mesh strips (Scrynel PET230 HC) are then laid down across the substrate in lines, covering the reactions areas exposed by the windows in the dielectric. An 5 mm wide polyester strip (50 microns thick) is then applied over the top of the mesh strips, and the edges of the electrodes are heat sealed. Finally, the substrate is cut up to provide 50 individual electrodes, for example having a size of 5.5 mm wide and 30 mm long.

EXAMPLE 3

30 Test strips manufactured using the ink formulation of Example 1 in the manner described in Example 2 were placed in a test meter with an applied voltage of

lines are essentially identical (0.1068 at 20 °C versus 0.1009 at 37 °C), thus demonstrating that the test strips provide essentially temperature-independent behavior over a temperature range from ambient to physiological temperatures.

EXAMPLE 6

The current transient was measured for a test strip prepared in accordance with Example 2 and for a commercial test strip made with a carbon-containing ink. The results are shown in Figs. 8A and 8B. As shown, the test strip of the invention (Fig. 8A) provides a very flat transient which maintains more than 50% of the peak current for a period of more than 25 seconds after the initial response from the test strip. In contrast, the carbon-based electrode exhibited an almost immediate decay in the current, having lost 50% of the peak current in a period of the first 1 to 2 seconds after the initial response from the test strip. This makes timing of the measurement difficult if peak current values are to be captured, or reduces the dynamic range of the meter if current must be measured after substantial decay has occurred. Thus, the test strips of the invention are advantageous in that the current generated in response to a given amount of glucose decays by less than 50% in the 5 seconds following peak current generation.

EXAMPLE 7

An ink for printing glucose test strips in accordance with the invention was formulated as follows:

67.8 g 20 mM Citrate buffer, pH 6
0.68 g Polyvinyl alcohol (MW 85,000-146,000, 88% hydrolysed)
0.68 g of Polyvinyl pyrrolidone-vinyl acetate
0.42 g of Dow Corning DC1500 antifoam
3.4 g of hydroxyethyl cellulose (Natrosol 250G, Hercules)
5.5 g of surface modified silica (Cabo-Sil TS 610, Cabot)
1.5 g glucose oxidase
20.0 g Potassium Ferricyanide.

Component	Amount
Analar Water	3L
Tri-sodium Citrate	15.75g
Nat 250 G	150g
Citric Acid	6.3g
Poly Vinyl Alcohol	30g
DC 1500 Defoamer	15 ml
Cabosil	225 g
Glucose Oxidase	48g
Potassium Hex/60299	660g
PVPVA	30g

After the integrated reagent/blood separation layer 110 is formed, a layer of mesh 111 is deposit over the sample collection region of the test strip. (Fig. 10E) The mesh 111 is preferably a nylon mesh which has been pretreated with acetone and Fluorad FC 170C surfactant to render the mesh hydrophilic. The purpose of the mesh 111 is the transport of the liquid sample evenly through the area between the working and reference electrodes.

A second insulation print 112 is then carried out using a slightly more flexible insulation ink (ERCON Insulayer 820202) to define the sample collection region. (Fig. 10F). A tape cover 113 is then applied over the top of the test strip as described above in Example 2 to form a finished test strip. (Fig. 10G).

1 3. The test strip of claim 2, wherein the matrix comprises silica
2 having both hydrophobic and hydrophilic surfaces.

1 4. The test strip of claims 3, wherein the first and second
2 conductive elements comprise conductive carbon.

1 5. The test strip according to claim 4, wherein the enzyme is
2 glucose oxidase.

1 6. The test strip according to claim 5, wherein the redox mediator
2 is ferricyanide.

1 7. The test strip according to claim 3, wherein the integrated
2 reagent/blood separation layer is formed from an aqueous composition comprising 2
3 to 10 % by weight of a binder; 3 to 10 % by weight of silica; 8 to 20 % by weight of
4 the redox mediator; and 1000 to 5000 units of the enzyme per gram of the aqueous
5 composition.

1 8. The test strip of claims 7, wherein the first and second
2 conductive elements comprise conductive carbon.

1 9. The test strip according to claim 7, wherein the enzyme is
2 glucose oxidase.

1 10. The test strip according to claim 9, wherein the redox mediator
2 is ferricyanide.

1 11. A method for forming a disposable test strip for use in a test
2 meter of the type which receives a disposable test strip and a sample of blood and
3 performs an electrochemical analysis of the amount of a blood analyte in the sample,
4 comprising:

1 17. The method of claim 16, wherein the enzyme is glucose
2 oxidase.

1 18. The method of to claim 17, wherein the redox mediator is
2 ferricyanide.

1 19. The method of claim 13, wherein the integrated reagent/blood
2 separation layer is formed from an aqueous composition comprising 2 to 10 % by
3 weight of a binder; 3 to 10 % by weight of silica; 8 to 20 % by weight of the redox
4 mediator; and 1000 to 5000 units of the enzyme per gram of the aqueous composition.

1 20. The method of claims 19, wherein the first and second
2 conductive elements comprise conductive carbon.

1 21. The method of claim 19, wherein the enzyme is glucose
2 oxidase.

1 22. The method of claim 21, wherein the redox mediator is
2 ferricyanide.

1 23. The method of claim 13, wherein the insulation layer is formed
2 over both the first and second conductive elements, and includes a second aperture
3 aligned with the second conductive element.

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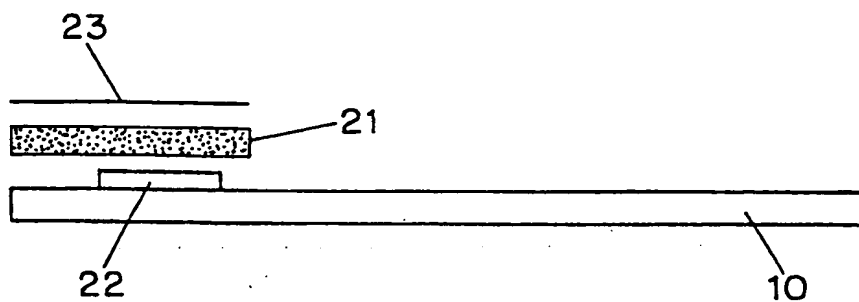


FIG. 2

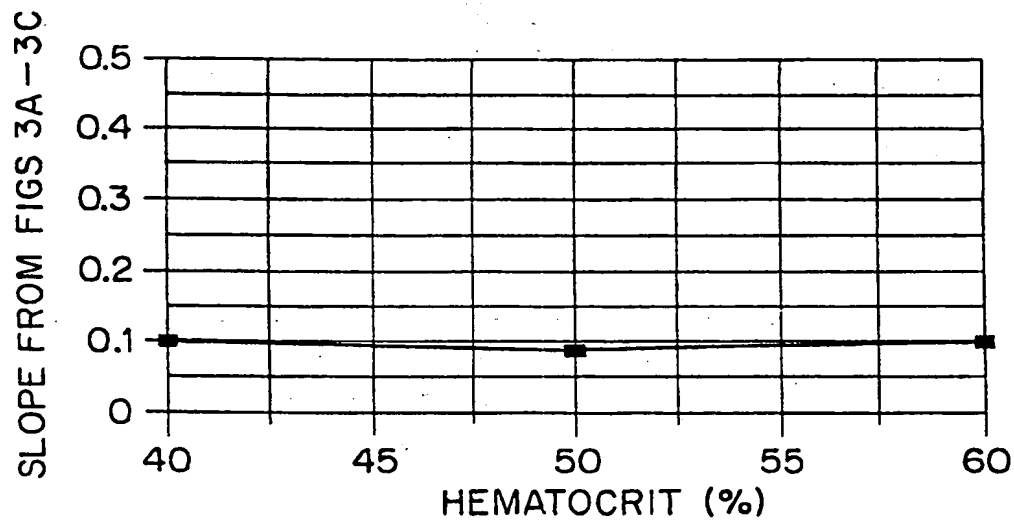


FIG. 4

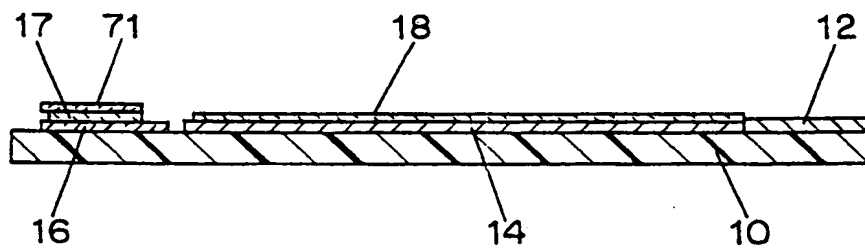


FIG. 7

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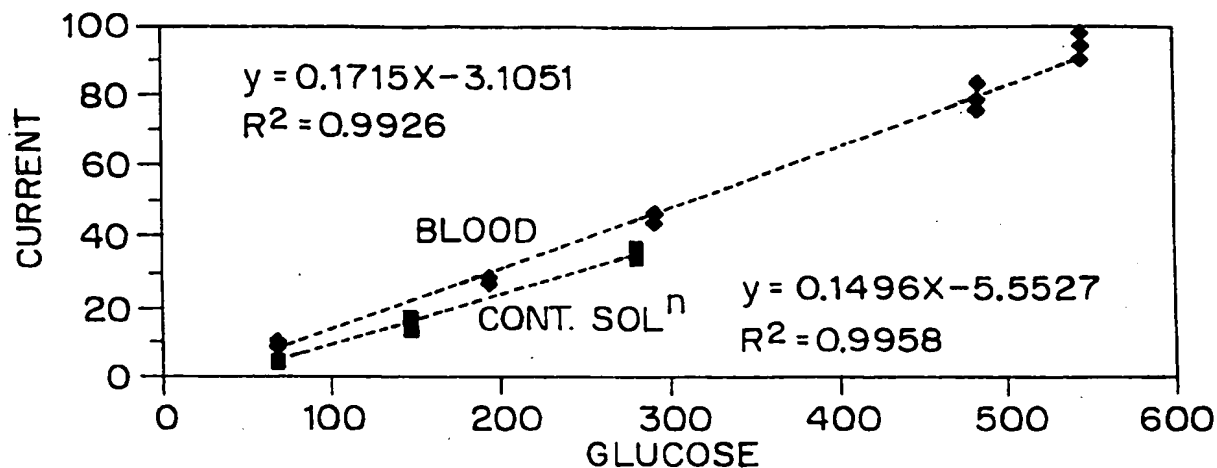


FIG. 5A

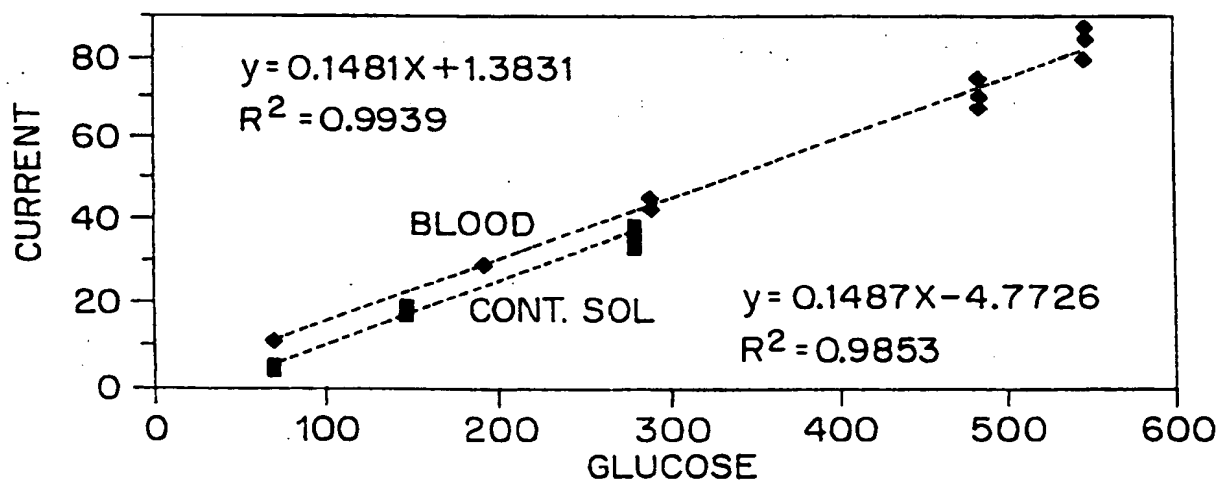


FIG. 5B

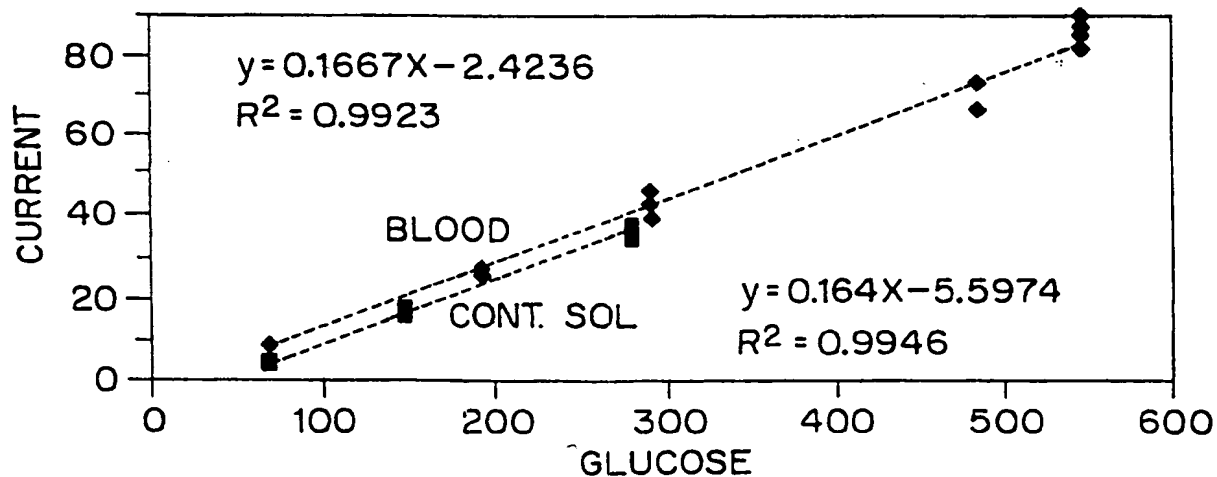


FIG. 5C

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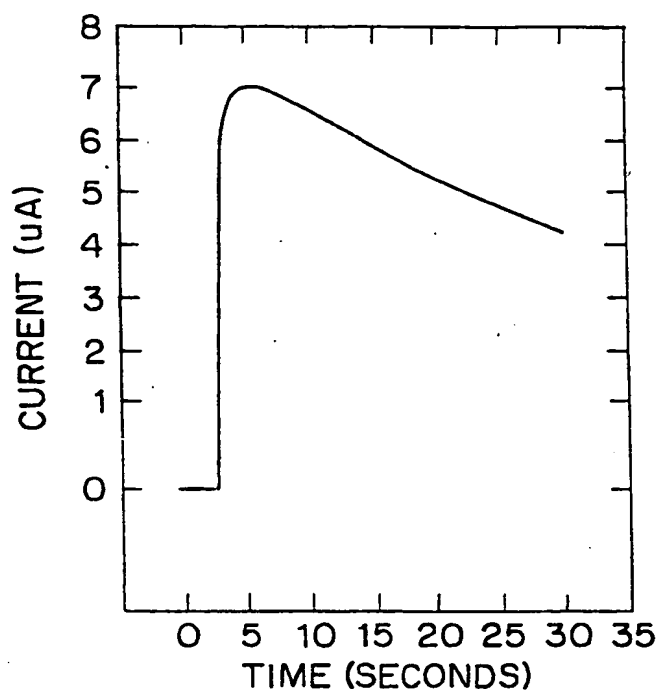


FIG. 8A

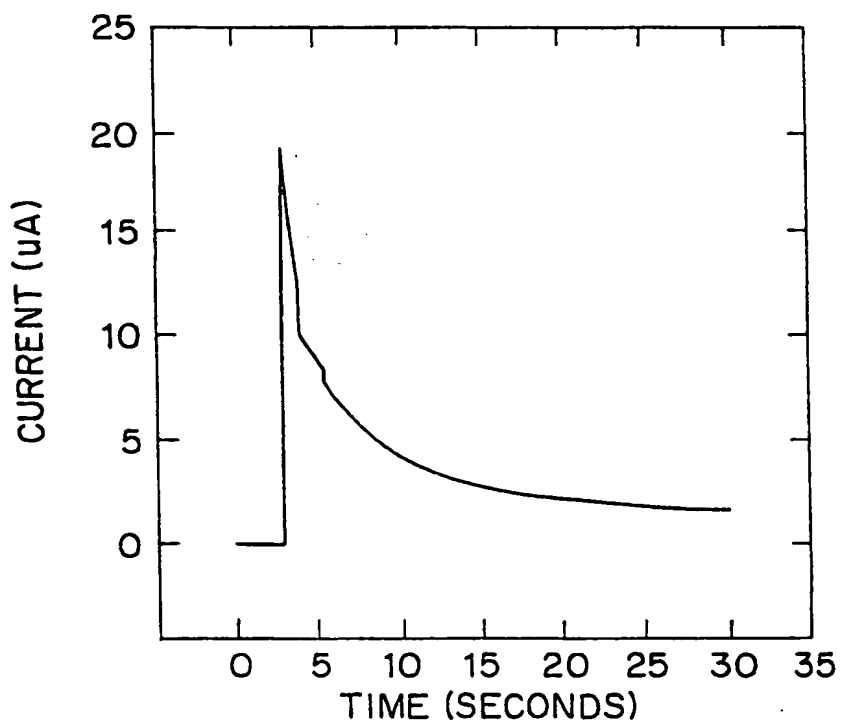


FIG. 8B

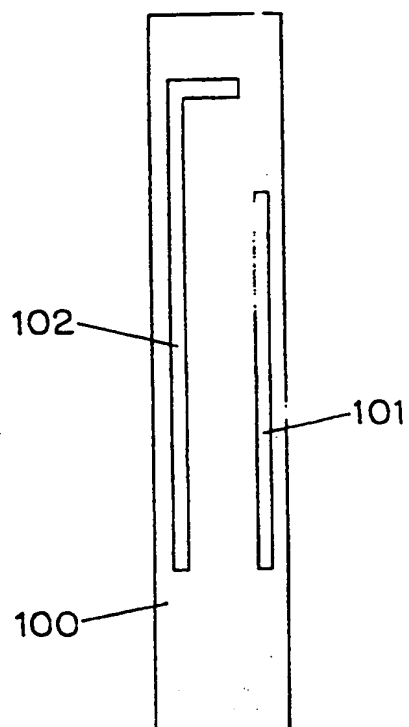


FIG. 10A

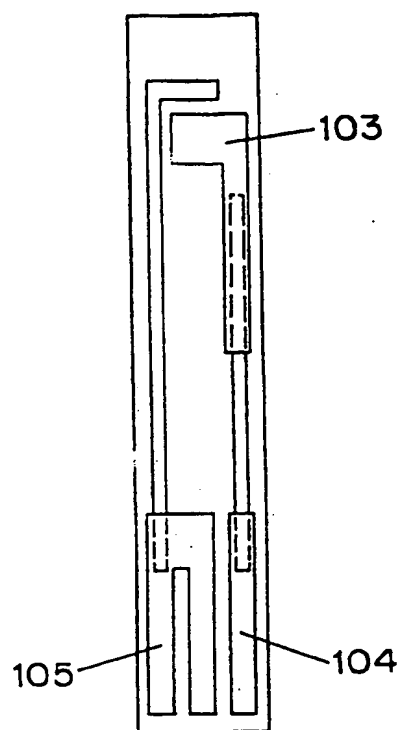


FIG. 10B

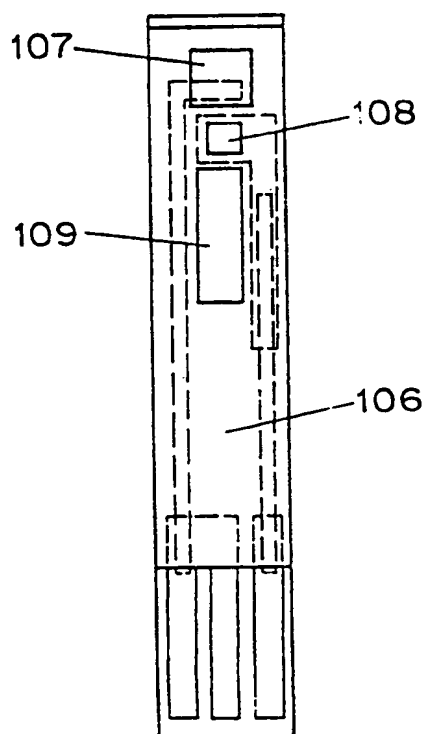


FIG. 10C

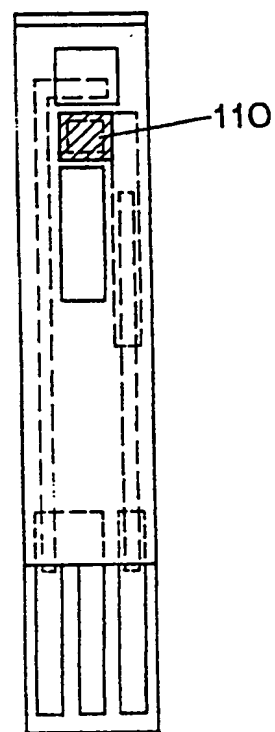


FIG. 10D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/00620

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 27/26

US CL : 204/403

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/403, 415; 205/777.5, 778; 427/2.11, 2.12, 2.13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS

search terms: sensor, detector, test strip, oxidase, glucose, blood, silica?, cab-o-sil

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0207370 A2 (JONES) 07 January 1987, the abstract; claims 2, 5, and 8; and page 6, line 5 - page 7, line 29.	1-23
Y	US 5,185,256 A (NANKAI et al.) 09 February 1993, the abstract and Figures 3 and 8.	1-23
Y	US 5,601,694 A (MALEY et al) 11 February 1997, the abstract; Figures 9A and 9B; col. 17, ll. 46-63; col. 19, ln. 34-45; col. 22, ll. 43-48; and col. 17, ln. 46 - col. 18, ln. 46.	1-23

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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B earlier document published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z

document member of the same patent family

Date of the actual completion of the international search

30 MARCH 2000

Date of mailing of the international search report

25 APR 2000

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